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Antioxidant, antibacterial and muscle relaxant activity of hydro-alcoholic leaf extract of *A. marmelos* and *J. zeylanica*

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Abstract
Different concentration of hydro-alcoholic leaf extracts of *Aegle marmelos* and *Justicia zeylanica* were investigated for *in vitro* antioxidant (at concentration of 10-1000 µg/ml) and antibacterial (at concentration of 100, 150, 200 and 500 mg/ml) activity. Further, *in vivo* muscle relaxant and grip strength activities (at concentration of 100 and 200 mg/ml) were also investigated. Both leaf extracts show antioxidant activity (such as total phenolic content, DPPH, ABTS, FRAP assay) as compared to standard but *J. zeylanica* showed more antioxidant potential as compared to *A. marmelos*. Both the extract show antibacterial activity against different bacterial strains (*S. aureus, B. cereus, L. monocytogenes, E. coli, S. typhimurium, Y. enterocolitica, V. cholera* and *S. flexneri*). However, present study revealed no muscle relaxant and grip strength activity of hydro-alcoholic leaf extracts of *A. marmelos* and *J. zeylanica* as when compared to the control group.

Keywords: *Aegle marmelos, Justicia zeylanica,* antioxidant, antibacterial, muscle relaxant, grip strength

1. Introduction
Plants form the backbone of the traditional system of medicine in India and are a potential source of bioactive compounds. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many of them based on their use in traditional medicine (Boominathan and Ramamurthy, 2009) [6]. It is documented that 80% of the world's population has faith in traditional medicine, particularly plant-based drugs for their primary healthcare. It is generally estimated that over 6000 plants in India are in use as traditional, folk, and herbal medicine, representing about 75% of the medicinal needs of developing countries (Kukreja and Maclaren, 1999) [27]. It has been documented that about 25% of the medicines prescribed globally are derived from herbal sources and about 121 phytoconstituents are in clinical use (Sahoo *et al*., 2010) [41]. Even in the essential medicines list given by the World Health Organisation (WHO), 11 % are exclusively planted derivatives (Rates, 2001) [38]. The World Health Organization (WHO) has listed 21,000 plants that are used for medicinal purposes around the world. Among these, 2,500 species are in India out of which 150 species are used commercially at a large scale. India is the largest producer of medicinal herbs and it is called as Botanical garden of the world (Bailey *et al* 1989) [4].

Aegle marmelos (L.) (AM) Corr. belongs to Rutaceae family, commonly known as Bael, is a Sub-tropical plant with short and thick trunk, soft and flaking bark, and native to indo-Malayan region (Hooker, 1975) [18] mainly India, Pakistan, Bangladesh, Sri Lanka, Burma, and Thailand (Islam *et al.*, 1995) [19]. This plant has also been mentioned in ancient Indian scriptures like Yajurveda and Mahabharata (Asha & Krishan, 2016) [2]. The Bael (AM) has vast therapeutics properties due to presence of mainly alkaloids, cardiac glycoside, saponin, steroids, coumarins (marmelosin, marmesin, marmin, imperatorin, scopoletin), Limonene, terpenoids (α-Phellandrene) (Asha and Krishan, 2016) [2], phenylpropanoids, tannins (skimmianine), polysaccharides, flavonoids (rutin flavon and flavonols) (Neeraj and Johar, 2017) [34] and α-glucosidase inhibitors (anhydromarmeline, aegelinosides A and B) (Tuticorin and Manakkal, 1983) [50]. Aeglin, rutin, γ-sitosterol, lupeol, marmesinin, β-sitosterol, flavone, glycoside, and phenylethyl cinnamamides are all found in the leaves of *Aegle marmelos*. The bael leaves are used to cure wound, leucorrhoea, conjunctivitis, jaundice, deafness, ulcer, cholera, hypoglycemia, asthma, hepatitis and the use of fruit as carminative, astringent, and

has good utility in thyroid disorder (Patel *et al.*, 2012) [35].
Justicia zeylanica (L.) (JZ) Nees belongs to Acanthaceae family, commonly known as Adulasa, is a small, sub herbaceous perennial shrub found in many regions of India and throughout the world (Cleason *et al.*, 2000) [8]. The plant has been employed in India's traditional medical system (Ayurvedic and Unani medicine) for more than 2000 years (Atal, 1980) [3]. The Adulasa (JZ) has vast therapeutics properties due to presence of mainly many pyrroquinazoline (bitter) alkaloids *viz*. vasicine (approx 0.0541 to 1.2 percent), vasicol, vasicinone, along with minor constituents such as adhatonine, vasicinol and vasicinolone. Alkaloids isolated from leaves are vasicoline, adhatodine, vasicolinone, and anisotine. Other than this, some steroids (daucosterol), flavonoids (astragalin, quercetin), triterpenes (a-amyrin), and alkanes have been found (Iyengar *et al.*, 1994[20]; Rawat *et al.*, 1994[38]; Singh 1997[44]). Adulasa leaves are used to treat cold, cough, chronic bronchitis, and asthma and it is also used as a sedative, expectorant, and antispasmodic (Kumar *et al* 2014[28]). The plant has antispasmodic, antihelminthic, insecticidal, and parasiticidal properties (Gulfraz *et al* 2004[15]). Hence the present study was undertaken to evaluate the *in vitro* antioxidant, antibacterial and *in vivo* muscle relaxant and grip strength effects of hydro-alcoholic leaf extract of *Aegle marmelos* and *Justicia zeylanica*.

2. Material and methods

2.1 Plant material

Based on ethno-pharmacological information, leaves were collected from campus of Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India and were authenticated by the botanist of Collaborative Ayurveda Research Centre, GADVASU, Ludhiana. Immediately after collection, leaves were washed and dried under sunlight. The dried leaves were finely grounded into powder, weighed and kept for further analysis.

2.2. Extraction

Hydro-alcoholic extract of the leaves were prepared using menstruum *viz*. ethanol and water (70:30) by maceration technique. 100 gram of powdered leaves was soaked in 1 litre menstruum at room temperature for 48-72 h and stirred at frequent time intervals. After maceration, the extract was initially filtered using muslin cloth and then re-filtered again using Whatman filter paper No 1. The filtrate obtained was evaporated in oven at a temperature of 40°C. The residue obtained was lyophilized and kept at 4°C in air tight bottles until used. Percent yield for Hydro-alcoholic extract was found to be 17.6±0.11 %, repectively.

2.3 Determination of Total Phenolic Content

The total phenolic content was estimated in the leaf extracts of AM and JZ using Folin-ciocalteu reagent (FCR) based assay (Singleton *et al*., 1999[46]) and absorbance was recorded at 725 nm. A standard curve of gallic acid was prepared (Fig.1). Total phenolic contents (mg/g) in the leaf extracts were expressed as gallic acid equivalent (GAE).

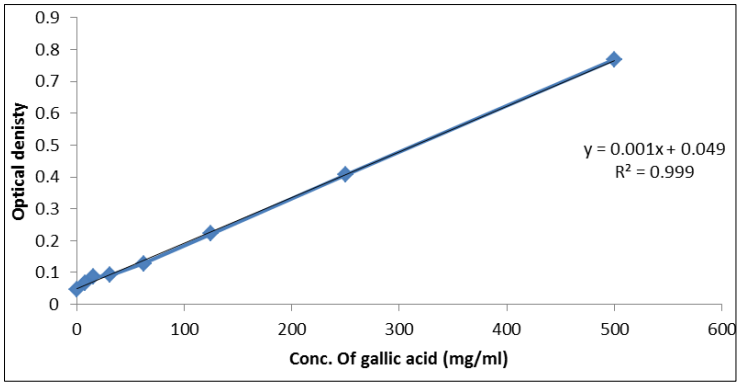

Fig 1: Standard curve of gallic acid for estimation of total phenols.

2.4 Evaluation of *in vitro* antioxidant activity

The total antioxidant activity of plant extract was determined by using ABTS (2,2´-azino-bis(3-ethylbenzothiazoline)-6-sulfonic acid) according to the method of Yilmaz *et al.* (2015) [52]. For total antioxidant activity, the trolox used as a standard and the concentration for extract and standard were ranging from 10 to 1000 µg/ml. The ability of extracts to scavenge DPPH (2, 2-diphenyl-1-picrylhydrazyl) radicals was determined according to the method of Brand-William *et al.* (1995) [7] and Yi *et al.* (2008) [51] and Butylated Hydroxytoulene (BHT) taken as standard. The ferric ion reducing antioxidant capacity of medicinal plants was determined by measuring the change in absorbance at 593 nm spectrophotometrically followed by the procedure of Benzie and Strain (1996) [5] which involved the reduction of Fe^{3+} 2,4,6 – Tris (2-pyridyl)-s- triazine (TPTZ) complex (colourless complex) to Fe^{2+} TPTZ (blue coloured complex) formed by the action of electron donating antioxidants at low pH.

2.5 Evaluatiom of *in vitro* antibacterial potential with well diffusion method

Hydro-alcoholic leaf extracts of both plants were evaluated for their antibacterial activity against gram+ve (*Bacillus cereus* (MTCC 6728), *Staphylococcus aureus* (MTCC 7443), *Listeria monocytogenes* (MTCC 657),) and gram-ve (*Escherichia coli* (MTCC 2991), *Salmonella typhimurium* (MTCC 3231), *Yersinia enterocolitica* (MTCC 3238), *Shigella flexneri* (MTCC 1457). These activities were compared with standard drug ampicillin and gentamicin against these organisms. Different concentrations (100, 200 and 500 mg/ml) of the plant extracts were dissolved in one percent dimethylsulphoxide (DMSO) for antimicrobial study.

2.6 Culture media and chemicals

Absolute alcohol, Nutrient broth (Merck specialities Private limited, Mumbai), Muller Hinton broth (Merck specialities Private limited, Mumbai), Muller Hinton agar (MHA), MacConkey agar (MLA) and Mannitol Salt agar (MSA)

(HIMEDIA) were commercially procured.

2.7 Well diffusion method assay

The agar well diffusion method (Collins *et al*. 1995) [9] was followed to test the antibacterial activity of different plant extracts. The sterilized media (MHA) was distributed into pre-sterilized petri plates (in triplicate) at a depth of approximately 4 mm (15 ml) and allowed to solidify. The medium of each plate was surface inoculated with a suspension of respective microorganisms (10^8cfu/ml) and the wells were cut using a sterile cork borer. The solution of various plant extracts (50 µl) in corresponding solvent at concentration of 100, 200 and 500 mg/ml was delivered into the wells. The solvent *i.e.* DMSO (50 µl), ampicillin (10 µg/ml) for gram+ve bacteria and Gentamicin (10 µg/ml) for gram-ve bacteria were maintained as negative and positive controls, respectively. The plates were incubated at 37°C for 24 h. following incubation the diameter of inhibition zones was recorded in millimeter (mm). The diameter of control or standardized zone was taken 5 mm.

2.8 *In vivo* Studies of the leaf extract

The present investigation was conducted on 36 female albino Sprague Dawley rats aged 2-3 months, weighing 100-150 g, at Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana. The animals were purchased from IMTECH Centre for Animal Resources and Experimentation (Icare), CSIR-IMTECH, Chandigarh India. The animals were acclimatized to the environment for two weeks before starting the experiment and kept in cages under standard laboratory conditions of temperature (27-30° C), with a 12 hour light cycle. Feed and water were provided *ad libitum* to the animals. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) vide reference no. GADVASU/2020/IAEC/55/20 dated 31.10.2020 and was conducted in accordance with ethical committee guidelines.

2.8.1 Muscle relaxant activity

A muscle relaxant is a drug that affects skeletal muscle function, decreases the muscle tone and used to alleviate symptoms such as muscle spasms, pain and hyper-reflexia. The test was done as per the method prescribed by Tirumalasetti *et al* (2015) [49]. In the study, the animals were trained to maintain balance for 40-60 sec on the rota rod rotating at a speed of 25 rpm. Only those rats which could balance themselves for 45-60 seconds were selected for study. Each rat was placed on the rota rod and time spent on rota rod was noted. Group I served as control and received caboxy methyl cellulose (1% CMC) as vehicle orally. Group II was administered with standard drug diazepam (Neon laboratories Ltd., India) @ 5 mg/kg intraperitoneally. Group III and IV were administered with hydro alcoholic extract of leaves of AM @ 100 mg/kg and 200 mg/kg orally. Similarly, Group V and VI were administered with hydro alcoholic extract of leaves of JZ @ 100 mg/kg and 200 mg/kg orally.

2.8.2 Grip Strength

The grip strength of limbs or muscular strength was measured using Grip Strength Meter (UgoBasile, Milan, Italy). In this triangular shaped metallic grip was attached to the transducer that measured the force which in turn was connected to a computer to digital signal. The Grip-Strength Meter automatically measures grip strength (i.e. peak force and time resistance) of fore limbs and also via the optional grid of hind limbs in rats. The effects of drugs, toxins, muscle relaxants, disease, ageing or neural damage on muscle strength may be assessed. After adequate training of the animals to hold the grip, animal was pulled back at increasing force holding the abdomen and tail, and the rats ability to hold on triangular holder was measured in GF (Grip force) unit. The trial was repeated thrice in order to avoid the false reading and the highest force measured was considered for further evaluation. The test was done as per the method prescribed by Rojecky *et al*. (2005) [40].

3. Results

3.1 Measurement of total phenolic content

The antioxidant activities of phenolic compounds are mainly of redox properties, which include free radical scavenging, hydrogen donating and singlet oxygen quenching activities. They can protect the body from free radicals, the formation of which is associated with the normal natural metabolism of aerobic cells (Halliwell 1996) [16]. The total phenolic content is an indication of strong antioxidant activity (Kahkonen *et al*. 1999) [23]. The total phenolic content is determined using Folin-Ciocalteu reagent (FCR) based assay (Gulcin *et al*. 2002) [14] which is an electron transfer based assay and gives reducing capacity which is expressed as phenolic content. The total phenolic content in hydro-alcoholic leaf extract of AM was 77 ± 1.15 and in JZ was 26.42 ± 1.05 mg GAE/gm. Our results are in agreement with Purena *et al.*, (2018) [36] who reported that total phenolic in aqueous extract of bael leaves was 53.37 µg GAE/ mg and in hydro-ethanolic extract of bael leaves was 75.13 µg GAE/ mg. Similarly, Sikri and dalal (2018) [43] reported that the total phenolic in methanol and aqueous extract of *Justicia adhatoda* leaves were 18.91 ± 0.10 and 87.66 ± 0.34 mg GAE/gm.

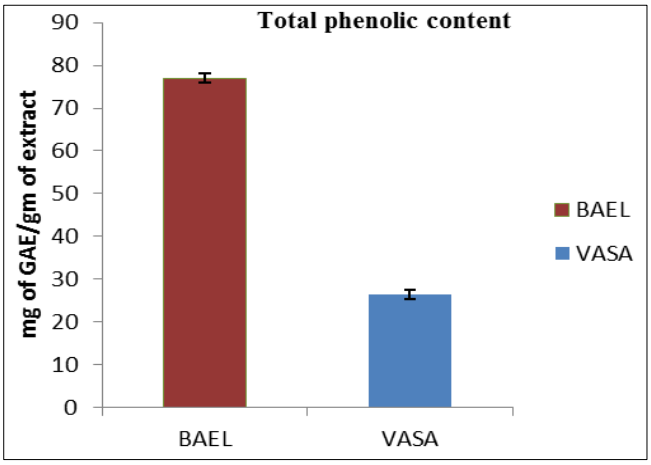

Fig 2: Total phenolic content of leaf extracts

3.2 Measurement of antioxidant activity

3.2.1 ABTS method

The total antioxidant activity of the hydro-alcoholic leaf extracts was measured by ABTS method. ABTS reacts with potassium persulphate to produce ABTS radical cation ($ABTS^+$), exhibiting a blue green chromogen with maximum absorption at 734 nm. The extent of decolorization is significant indicator of antioxidant activity of the sample. The effects of extracts on ABTS radical cation is due to their hydrogen donating ability which is visually observed by a change in the color of radical cation ($ABTS^+$) to colorless ABTS. Almost all the extracts were found to be effective in scavenging the ABTS radical. The antioxidant activity was

determined on the basis of IC_{50} value which is the concentration required for 50% inhibition of free radical. The percentage inhibition of ABTS radical was concentration-dependent. The ABTS scavenging activity of AM and JZ leaf extracts was found to be 101.46 and 90.39 (µg/ml) and that of the standard, trolox, was 66.49 (µg/ml) respectively. IC_{50} values for ABTS free radical scavenging of hydro-alcoholic leaf extracts showed lesser scavenging effect as compared to standard trolox. Our results are concurring with Rajamurugan *et al.* (2013) [37] who reported that the ethanloic extract of *A. marmelos* showed the total antioxidant activity, ABTS radical was effectively scavenged. IC_{50} value was 102 µg/ml which showed lesser scavenging effect than ascorbic effect (IC_{50} = 96 µg/ml). Maridonneau-Pairini *et al*. (1986) [30] reported that the presence of flavonoids such as leucoantocyanins, anthocyanins, and flavonoid glycosides in *A. marmelos* may be the primary cause of its activity. Similarly, Kaur *et al* (2015) [25] who reported that the n-butanol and ethyl acetate extracts of *J. zeylanica* exhibited the $ABTS^{+}$ scavenging ability and the % inhibition of these extracts were 92.48 and 91.29 %, respectively, where as gallic acid scavenged 93.86 % radicals at same concentration. The antioxidant property of leaves of *Justicia zeylanica* were due to quinazoline alkaloid (vasicine) which was isolated using gas chromatographic and mass spectrometric analysis (Kaur *et al*. 2016) [24]

Table 1: Total antioxidant activity (Mean±SE) of trolox and leaf extracts (n=3)

S. No.	Concentration (µg/ml)	% Inhibtion		
		Trolox	*Aegle marmelos*	*Justicia zeylanica*
1	1000	87.32 ± 0.72	77.56 ± 0.20	87.74 ± 0.12
2	800	80.24 ± 0.08	67.69 ± 0.17	73.42 ± 0.11
3	400	75.59 ± 0.26	54.75 ± 0.28	57.87 ± 0.07
4	200	69.77 ± 0.22	43.21 ± 0.28	43.80 ± 0. 08
5	100	65.17 ± 0.33	34.95 ± 0.23	31.50 ± 0.11
6	50	52.49 ± 0.29	28.57 ± 0.09	25.7 ± 0.18
7	25	39.83 ± 0.17	24.09 ± 0.37	20.53 ± 0.19
	IC_{50} (µg/ml)	66.49	101.46	90.39

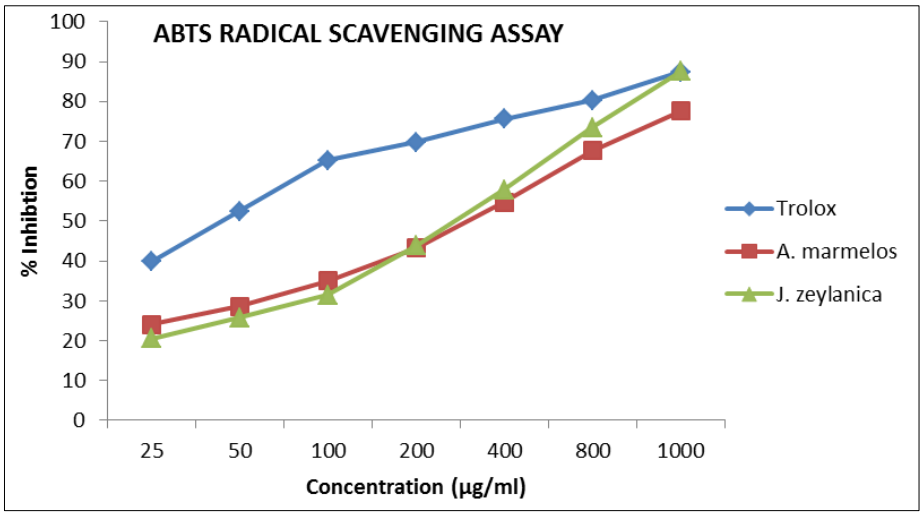

Fig 3: Total antioxidant activity of trolox and leaf extracts

3.2.2 DPPH Method

DPPH (2,2-diphenyl-1-picrylhydrazyl) stable free radical method is a sensitive way to determine the antioxidant activity of plant extracts. Antioxidants neutralize the free radicals on interaction with DPPH by transferring electrons or hydrogen atoms to DPPH (Archana *et al*. 2005) [1]. DPPH is a stable free radical that accepts free electron or hydrogen radical to become a stable diamagnetic molecule (Matthaus 2002) [31].These also include related reactive oxygen species (ROS) that leads to free radical generation, causing the cascading chain reaction in biological system. Antioxidants present in various dietary supplements offer their beneficial effects by neutralizing these ROS during various disease conditions. The radical scavenging activity, using a DPPH generated radical, was tested with different sample extracts along with butylated hydroxytoluene (BHT). The antioxidant activity was determined on the basis of IC_{50} value. The IC_{50} value for DPPH free radical scavenging of AM and JZ hydro-alcoholic leaf extracts were found to be 416.23 and 70.71 µg/ml and BHT was taken as standard antioxidant and IC_{50} value of BHT was found to be 23.73 µg/ml. Lower IC_{50} value showed higher antioxidant activity. Our results are concurring with Rajamurugan *et al.* (2013) [37] who reported that the ethanloic extract of *A. marmelos* showed the total antioxidant activity, ABTS radical was effectively scavenged. IC_{50} value was 102 µg/ml which showed lesser scavenging effect than ascorbic effect (IC_{50} = 96 µg/ml). Maridonneau-Pairini *et al*. (1986) [30] reported that the presence of flavonoids such as leucoantocyanins, anthocyanins, and flavonoid glycosides in *A. marmelos* may be the primary cause of its activity. Similarly for *Justicia zeylanica* Kaur *et al*. (2015) [25] who reported that the n-butanol and ethyl acetate exhibited the $ABTS^{+}$ scavenging ability and the % inhibition of these extracts were 92.48 and 91.29 %, respectively, where as gallic acid scavenged 93.86 % radicals at same concentration. The antioxidant property of leaves of *Justicia zeylanica* were due to quinazoline alkaloid (vasicine) which was isolated using gas chromatographic and mass spectrometric analysis (Kaur *et al.*, 2016) [24].

Table 2: Free radical scavenging activity (Mean ± SE) of BHT and leaf extracts (n=3)

S. No.	Concentration (µg/ml)	% Inhibtion		
		BHT	Aegle marmelos	*J. zeylanica*
1	1000	76.23 ± 0.72	66.90 ± 0.14	54.87 ± 0.14
2	800	72.95 ± 0.26	62.97 ± 0.17	50.69 ± 0.14
3	400	69.67 ± 0.70	54.77 ± 0.08	48.45 ± 0.22
4	200	69.39 ± 0.88	42.80 ± 0.28	39.67 ± 0. 09
5	100	59.84 ± 0.19	34.85 ± 0.20	35.35 ± 0.18
6	50	52.46 ± 0.13	26.89 ± 0.12	22.56 ± 0.12
7	25	34.43 ± 0.43	23.49 ± 0.20	16.64 ± 0.09
	IC_{50} (µg/ml)	23.73	416.23	70.71

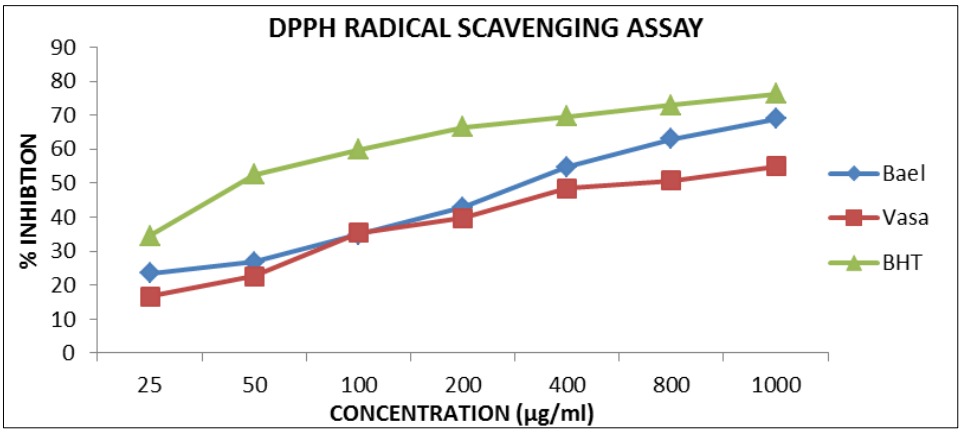

Fig 4: Free radical scavenging activity of BHT and leaf extracts

3.2.3 FRAP assay

FRAP assay measures the reducing potential of antioxidants. Antioxidant compound which acts as a reducing agent exerts its effects by donating hydrogen atom to ferric complex and thus, breaks the radical chain reaction (Singh and Rajini 2004[45]). The reducing capacity of ferric ion was found 184.34 and 84.36 (µg ofAA/ mg of dried extract) for *A. marmelos* and *J. zeylanica* and 25.25 (µg ofAA/ mg of dried extract) for standard ascorbic acid. Our results were in agreement with Jha and Gupta (2015) [21] who reported that the reducing capability of ferric ion in *Aegle marmelos* was found to be 268.58 µg BE/mg of sample. Similarly for *J. zeylanica* Giri *et al*. (2014) [13] determined the antioxidant activity of methnolic leaf extract of *J. adhatoda* which were evaluated by FRAP assay and found to be 0.794 mM Fe (II)/L.

Table 3: Ferric reducing antioxidant activity (Mean ± SE) of BHT and leaf extracts (n=3)

S. No.	Conc. (µg/ml)	Reducing power assay (µg of AA/ mg of dried extract)		
		Ascorbic acid	*A. marmelos*	*J. zeylanica*
1	1000	93.34 ± 0.04	62.60 ± 0.17	80.22 ± 0.11
2	800	87.43 ± 0.03	53.65 ± 0.06	69.34 ± 0.16
3	400	75.51 ± 0.05	39.52 ± 0.10	52.48 ± 0.10
4	200	65.48 ± 0.03	29.68 ± 0.14	37.57 ± 0. 09
5	100	59.39 ± 0.03	17.80 ± 0.10	26.82 ± 0.08
6	50	50.27 ± 0.04	6.82 ± 0.08	19.53 ± 0.12
7	25	42.56 ± 0.02	3.51 ± 0.09	10.39 ± 0.10
	IC_{50}	25.25	184.34	84.36

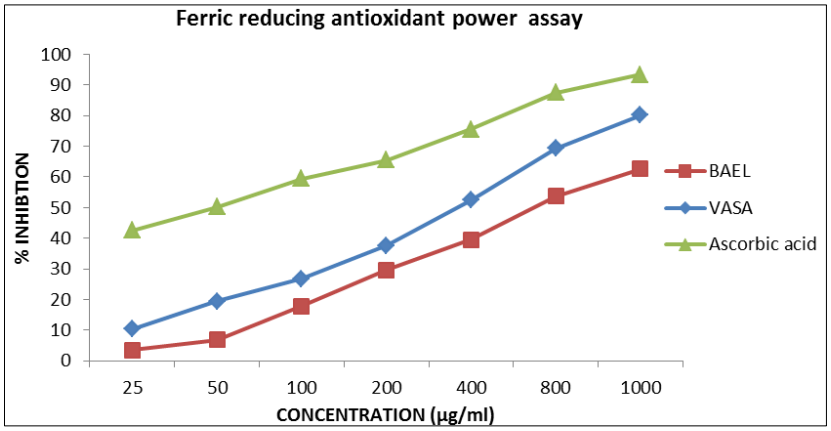

Fig 5: Ferric reducing antioxidant activity of BHT and leaf extracts

3.3 Antibacterial activity

The antibacterial activity of AM and JZ is presented in Table 4 & 5. The present investigation shows the efficacy of hydro-alcoholic leaf extract against the selected pathogenic bacteria. The maximum zone of inhibition was observed at the concentration of 500 mg/ml. varying degree of antibacterial activity by leaf extracts of AM and JZ against various tested bacterial species has been reported. The antibacterial activity of the AM leaf extracts may be due to the presence of cuminaldehyde and eugenol because these compounds have already shown their activities against various bacterial strains (Duke J A, 1992 [11]; Katayama T and Nagai I, 1960) [26]. Our findings are in agreement with Sivaraj *et al*. (2011) [47] who found the aqueous, petroleum ether and ethanol extract of the leaves of *A. marmelos* exhibited efficient antibacterial activity against *E. coli*, *S. pneuoniae*, *S. typhimurium*, *Klebisella pneumonia* and *Proteus vulgaris* and Mujeeb *et al.* (2011) [33] also found the ethanolic and methanolic extracts of leaves having antibacterial activities against *B. cereus*, *S. epidermis*, *S. aureus*, *E. aerogens*, *K. pneumonia*. Similarly, Maheshwari *et al*. (2009) [29] showed ethanolic extract of dried fruit pulp of *A. marmelos* against various intestinal pathogens i.e. *Shigella boydii*, *S. sonnei* and *S. flexneri*. Mith *et al*. (2014) [32] showed that the essential oils such as eugenol, carvacrol, trans-cinnamaldehyde, linalool and thymol having antibacterial properties against *L.monocytogenes*, *S. typhimurium*, *E. coli*, *B. thermospacta* and *P. fluorescens.* Similarly, the antibacterial activity of leaf extract of *J. zeylanica* was due to the compounds alkaloids (vasicine) (Duraipandiyan *et al* 2015) [12] and Adhavasinone (Ju *et al*. 2019) [22]. The results obtained in the evaluation of antibacterial activity of aqueous leaf extract of *J. zeylanica* against *S. aureus*, *V. cholorae*, *B. brevis*, *S. flexneri*, *E. coli*, *B. cereus*, *B. subtilis*, *B. licheniformis* and *p. aeruginosa* were reported by Thatoi *et al*. (2008) [48] and Sarker *et al*. (2009) [42].

Table 4: Zone of inhibition by hydro-alcoholic leaf extract of *A. marmelos* (AM)

Concentration (mg/ml)	100	150	200	500	Standard (10 µg/ml)	Control
Gram+ve bacteria		Zone of inhibition(mm)			Ampicillin	DMSO
S. aureus	9.83 ± 0.08^{a}	14.07 ± 0.12^{b}	15.93 ± 0.17^{c}	19.80 ± 0.11^{e}	17± 0.57^{d}	0
B. cereus	9.67± 0.88^{a}	12.73 ± 0.58^{b}	12.7± 0.89^{b}	16.87 ± 0.82^{c}	10.67 ± 0.67^{ab}	0
L. monocytogenes	----------	8.07 ± 0.07^{a}	11 ± 0.12^{b}	16.4± 0.11^{c}	20.33 ± 1.20^{d}	0
Gram-ve bacteria					Gentamicin	
E. coli	9.33 ± 0.03^{a}	10.13 ± 0.06^{ab}	11.27 ± 0.13^{c}	16.20± 0.11^{d}	16.67 ± 0.67^{d}	0
S. typhimurium	----------	----------	10.33 ± 0.33^{a}	13.8± 0.11^{b}	13.33 ± 0.67^{b}	0
Y. enterocolitica	----------	9.53± 0.29^{a}	10.67± 0.24^{a}	16.5± 0.55^{b}	19.33 ± 0.67^{c}	0
S. flexneri	11 ± 0.58^{a}	12.67± 0.67^{b}	14.33 ± 0.33^{b}	15.72± 0.58^{c}	13.33 ± 0.67^{b}	0
V. cholerae	10.8± 0.53^{a}	10.93 ± 0.24^{ab}	11.8 ± 0.11^{b}	15.4± 0.23^{c}	10.33 ± 0.33^{a}	0

Table 5: Zone of inhibition by hydro-alcoholic leaf extract of *J. zeylanica* (JZ)

Concentration (mg/ml)	100	150	200	500	Standard (10 µg/ml)	Control
Gram+ve bacteria		Zone of inhibition(mm)			Ampicillin	DMSO
S. aureus	15.80 ± 0.11^{a}	18.13 ± 0.07^{ab}	18.80 ± 0.11^{b}	22 ± 0.23^{c}	18.67 ± 0.67^{b}	0
B. cereus	----------	10.73 ± 0.17^{a}	10.8 ± 0.42^{a}	13.6 ± 0.35^{b}	10.33 ± 0.33^{a}	0
L. monocytogenes	10.33 ± 0.67^{a}	12.33 ± 0.82^{ab}	13.21 ± 0.32^{ab}	16 ± 1.12^{c}	21.33 ± 1.67^{d}	0
Gram-ve bacteria					Gentamicin	
E. coli	18.33 ± 0.18^{b}	20.67 ± 0.33^{c}	23.47 ± 0.07^{d}	26.1 ± 0.07^{e}	16.67 ± 0.67^{a}	0
S. typhimurium	10.80 ± 0.61^{a}	13.5 ± 0.29^{b}	13.53 ± 0.29^{b}	18.5 ± 0.68^{c}	13.33 ± 0.67^{b}	0
Y. enterocolitica	----------	----------	----------	13.3 ± 0.17^{a}	19.33 ± 0.67^{b}	0
S. flexneri	----------	9.70 ± 0.43^{a}	11.8 ± 0.46^{b}	14.8 ± 0.46^{c}	10.33 ± 0.33^{a}	0
V. cholerae	11.33 ± 0.33^{a}	15.67 ± 0.33^{c}	16 ± 0.57^{c}	20 ± 0.58^{d}	13 ± 0.57^{b}	0

Means bearing superscripts ^{a,b,c,d} differ significantly (p<0.05) in a column.

3.4 Muscle relaxant activity

Skeletal muscle relaxants are the agents which reduce the muscle tone. Skeletal muscle relaxants act either peripherally at the neuromuscular junction (neuromuscular block) such as d-tubocurarine and acetylcholine or centrally in the cerebrospinal axis such as diazepam or directly on the contractile mechanism of the muscle as with dantrolene. In the present study, the muscle relaxant activity of hydro-alcoholic leaf extracts of AM and JZ was evaluated in albino rats given in table 6 and Figure 6. Hence, present study showed no muscle relaxant activity of *A. marmelos* and *J. zeylanica* extracts. In contrast to present study, Hema *et al*. (1999) [17] studied the effect of the aqueous, alcoholic and petroleum ether extracts of *A. marmelos* at the dose of 500 mg/kg for the hypoglycaemic and other pharmacological actions and observed that the aqueous extract acts as a cardiac stimulant, smooth-muscle relaxant and uterine stimulant while the alcoholic extract revealed cardiac depressant, smooth muscle relaxant and uterine relaxant properties. Also, Cruz *et al*. (1999) [10] reported that the essential oil from the leaves of *J. adhatoda* showed smooth muscle relaxant activity in the isolated guinea-pig tracheal chain.

Table 6: Effect of hydro-alcoholic leaf extracts on muscle relaxant activity

Group	Dose (mg/kg)	Fall off time (sec)		
		0 h	1 h	2 h
Group 1 (CMC)	1%	31.67±0.95^{a}	34.14±1.25^{b}	33.68±0.97^{b}
Group II (Diazepam)	5	34.68±1.14^{a}	7.32±0.54^{a}	4.09±0.35^{a}
Group III (*A. marmelos)*	100	34.01±0.32^{a}	32.13±1.08^{b}	31.85±1.65^{b}
Group IV (*A. marmelos*)	200	33.69±0.39^{a}	33.80±0.87^{b}	32.16±1.15^{b}

Group V (*J. zeylanica*)	100	33.89±0.19[a]	32.93±0.59[b]	31.17±1.42[b]
Group VI (*J. zeylanica*)	200	34.42±0.26[a]	34.60±0.49[b]	32.12±0.90[b]

Values lacking a common superscript in given column differ significantly from each other (p<0.05).

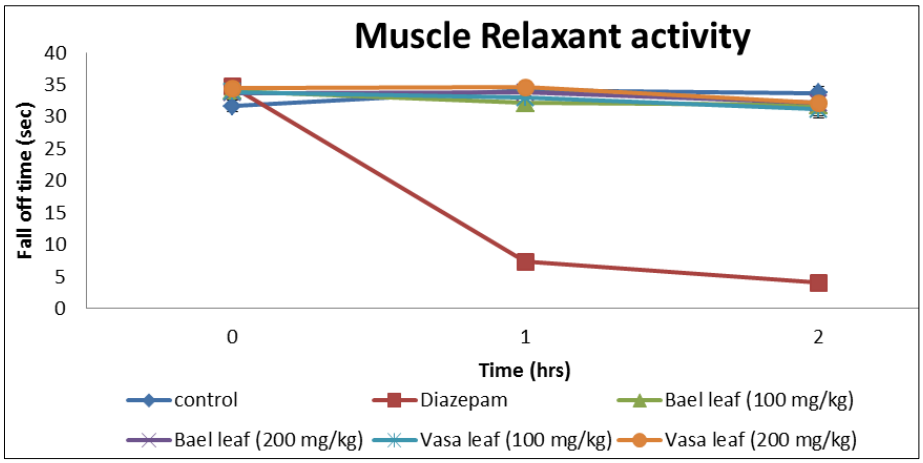

Fig 6: Effect of leaf extract on muscle relaxant activity in rats.

3.5 Grip strength activity

The grip strength is used for access the grip strength of limbs or muscular strength. The activity was measured using Grip strength meter. In the present study, the grip strength activity of hydro-alcoholic leaf extracts was evaluated in albino rats given in Table 7 and Figure 7. In Group II (standard group) there was significant (p<0.05) decrease in grip strength after 30 min which remained upto 1h after drug administration as compared to the control group. Hence, present study showed no grip strength activity in both leaf extracts.

Table 7: Effect of hydro-alcoholic leaf extracts on grip strength activity.

Group	Dose (mg/kg)	Grip Force			
		0 min	30 min	45 min	60 min
Group 1 (CMC)	1%	428.99±12.02[b]	430.07±9.35[c]	450.43±12.46[c]	453.53±11.64[c]
Group II (Diazepam)	5	440.30±2.93[c]	197.39±3.62[a]	103.29±5.06[a]	99.64±1.51[a]
Group III (AM)	100	416.84±5.65[ab]	406.13±4.22[b]	397.18±6.90[b]	389.64±10.19[b]
Group IV (AM)	200	406.21±1.74[a]	402.69±1.24[b]	397.73±1.30[b]	400.88±0.84[b]
Group V (JZ)	100	404.02±3.34[a]	408.18±1.54[b]	406.37±1.47[b]	403.08±1.24[b]
Group VI (JZ)	200	407.75±1.80[a]	404.86±2.07[b]	405.37±2.42[b]	404.60±1.27[b]

Values lacking a common superscript in given column differ significantly from each other (p<0.05).

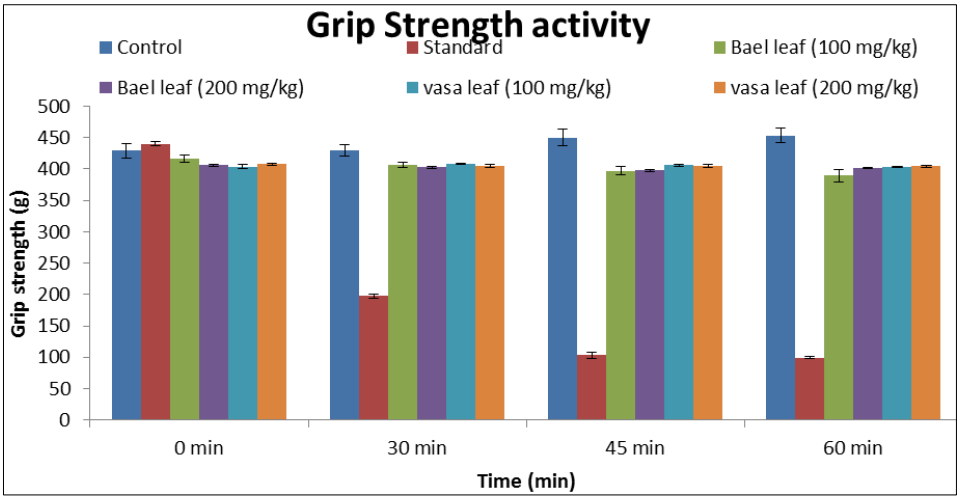

Fig 7: Effect of hydro-alcoholic leaf extracts on grip strength activity.

4. Summary and Conclusion

The present study was undertaken to study the antibacterial, antioxidant, muscle relaxant and grip strength activity of the leaf extracts of *Aegle marmelos* and *Justicia zeylanica*. The antioxidant and antibacterial potential of hydro-alcoholic leaf extract of *Aegle marmelos* and *Justicia zeylanica* was determined *in vitro*.

The hydro-alcoholic leaf extract of *J. zeylanica* showed higher total phenolic content (in terms of gallic acid equivalent), ABTS radical scavenging activity, DPPH free radical scavenging activity and FRAP assay (in terms of IC_{50}) as compared to *A. marmelos*. Study revealed the extracts of *A. marmelos* and *J. zeylanica* having antibacterial activity against *S. aureus*, *E. coli*, *B. cereus*, *L. monocytogenes*, *S. typhimurium*, *Y. enterocolitica*, *V. cholerae* and *S. flexneri* at concentrations of 100, 150, 200 and 500 mg/ml. Ampicillin

(for Gram +ve) and Gentamicin (for Gram -ve) were used as standard drugs at concentrations of 10 µg/ml and DMSO served as control. There was significant zone of inhibition in extracts of both plants as compared to control. Pharmacological potential of extracts was evaluated by conducting various experiments i.e. muscle relaxant activity and grip strength. Present study revealed no muscle relaxant and grip strength activity of hydro-alcoholic leaf extract of *A. marmelos* and *J. zeylanica*.

Hence, it can be concluded from the present study that the hydro-alcoholic leaf extracts of plants *Aegle marmelos* and *Justicia zeylanica* have the potential to be developed as the antioxidant and antibacterial effects after further investigations and separation of the active compounds.

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